



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Everything You Wanted to Know About Antimicrobial Susceptibility Testing (AST) of *Staphylococcus aureus*!

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**currently working with CDC's Division of Laboratory
Systems through an Interagency Personnel Agreement*

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**At the conclusion of this presentation,
you will be able to.....**

- ◆ **Describe current NCCLS recommendations for disk diffusion and MIC testing of *S. aureus*.**
- ◆ **Discuss current strategies for reporting AST results on *S. aureus*.**
- ◆ **List steps for verifying and reporting VISA and VRSA.**



**At the conclusion of this presentation,
you will be able to..... (con't)**

- ◆ List **supplemental drugs** that might be reported on MRSA, VISA, and VRSA and discuss testing of these agents.
- ◆ Describe a method for **assessing competency** of staff in detecting and reporting MRSA, VISA and VRSA.



Acronyms

MRSA – methicillin-resistant *S. aureus*

ORSA – oxacillin-resistant *S. aureus*
(**MRSA** = **ORSA**)

BORSA – borderline ORSA

CA-MRSA – community-associated MRSA

VISA – vancomycin-intermediate *S. aureus*

VRSA – vancomycin-resistant *S. aureus*

PBP2' (PBP2a) – penicillin-binding protein 2' (2a)

Staphylococcus aureus - Rx

Organism	1st Choice Drugs	Alternative Drugs
Oxacillin-S	oxacillin, nafcillin	a cephalosporin, vancomycin, β -lac/ β -lac inhibitor combo, carbapenem, macrolide, clindamycin, linezolid, quin-dalfo, fluoroquinolone
Oxacillin-R	vancomycin	linezolid, quin-dalfo, fluoroquinolone, trim-sulfa

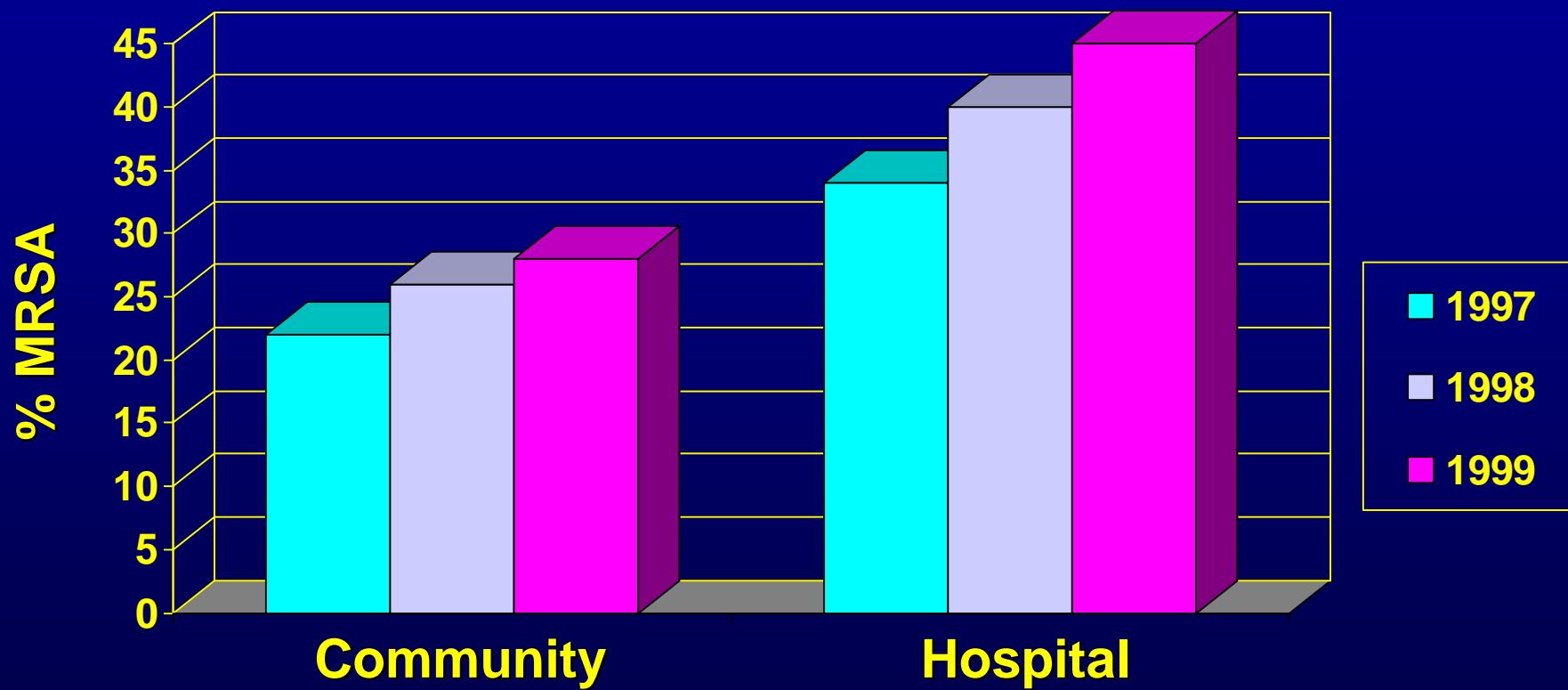
Sanford Guide, 2003.

MRSA Epidemiology

Methicillin-resistant *Staphylococcus aureus*

MRSA

S. aureus Bloodstream Infections



Diekema et al. 2001. Clin Infect Dis. 32(Suppl 2):S114

MRSA in Non-Healthcare Settings

Community Associated MRSA (CA-MRSA)

- ♦ Competitive sports
- ♦ School children
- ♦ Correctional facilities
- ♦ Men who have sex with men (MSM)

Salgado et al. 2003. Clin Infect Dis. 36(Suppl. 2):S131.
MMWR. 2003; 52:88.

CA-MRSA (con't)

- ◆ May possess **Panton Valentine leucocidin (PVL)**
 - Facilitates MRSA crossing intact skin barrier
 - Can cause septicemia in immunocompetent patients
 - Associated with lethal necrotizing pneumonia

CA-MRSA (con't)

- ◆ MRSA carry staphylococcal cassette chromosome *mec* (*SCCmec*) as methicillin resistant determinant
- ◆ Novel *SCCmec* (Type IV) in some CA-MRSA
- ◆ CA-MRSA may have distinct origin of derivation

CA-MRSA (con't)

- ♦ Often susceptible to:

clindamycin

erythromycin

fluoroquinolones

linezolid

rifampin

tetracyclines

trimeth-sulfa

vancomycin

S. aureus
Testing / Reporting

Test/Report

NCCLS Antimicrobial Susceptibility Testing (AST) Standards

- ◆ Instructions for performing test (2003)
 - M2-A8 Disk Diffusion
 - M7-A6 MIC
 - (Updated every 3 years)
- ◆ M100-S13 (2003) “The Tables”
 - Drugs to test/report
 - Interpretive Criteria (breakpoints)
 - Quality Control ranges
 - (Updated annually)



NCCLS AST Standards

- ◆ Describe “reference methods”
- ◆ Clinical labs can use:
 - NCCLS methods as written OR
 - Method that performs comparably to NCCLS “reference method” (e.g. FDA-cleared diagnostic device)

Vitek

MicroScan

Etest

Other

***S. aureus* - Oxacillin Special Testing Concerns**

Inoculum:

**McFarland 0.5 suspension
from fresh colonies (direct
colony suspension method)**

Incubation:

35°C; 24h

Disk diffusion (DD):

MHA*
**oxacillin (1 mg)
transmitted light**

CLIC testing:

CAMHB + 2% NaCl**

agar screen:

**MHA + 4% NaCl + 6 mg/ml
oxacillin**

***Mueller-Hinton agar**

****Cation-adjusted Mueller-Hinton broth**

Penicillinase-Labile Penicillins

- ♦ Include:

- amoxicillin**

- penicillin**

- ampicillin**

- piperacillin**

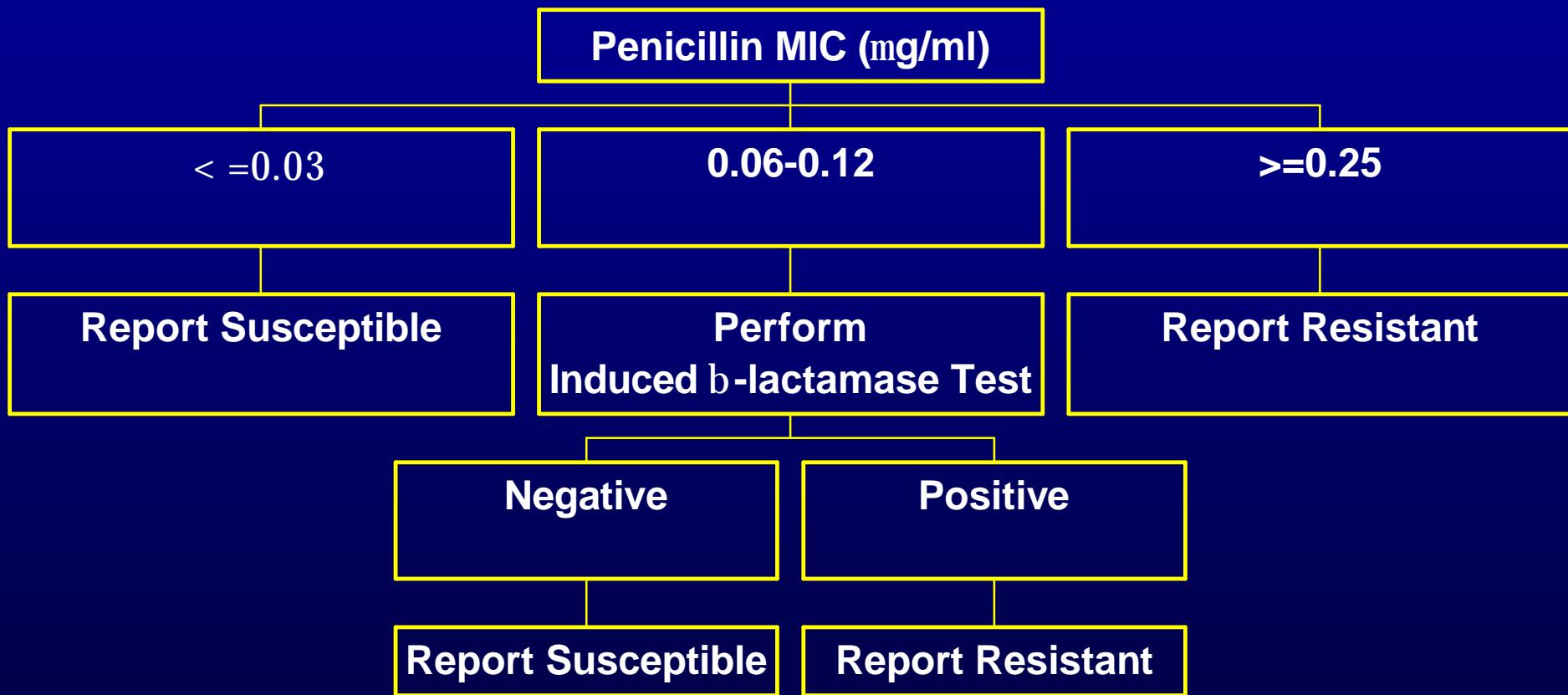
- carbenicillin**

- ticarcillin**

- mezlocillin**

- ♦ Inactivated by staphylococcal β -lactamase (penicillinase)
- ♦ **β -lactamase positive** = resistant to agents listed

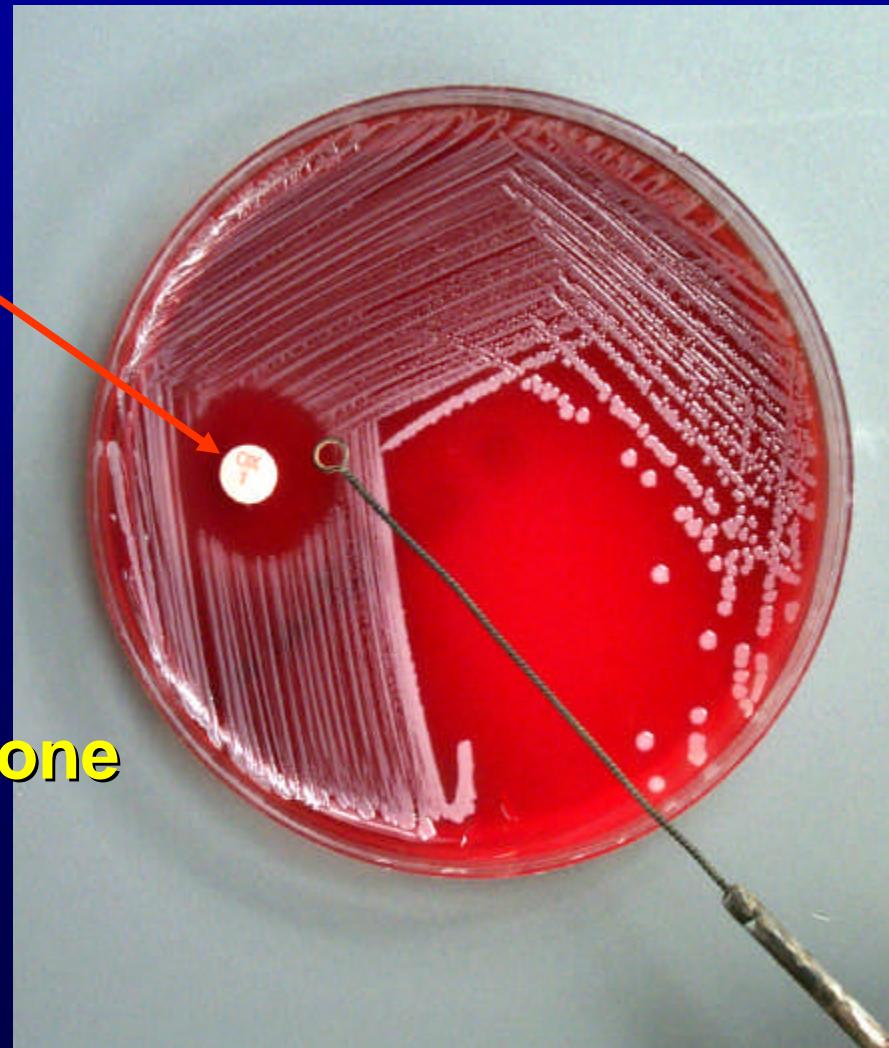
Staphylococcus spp. Penicillin “MIC” Testing/Reporting



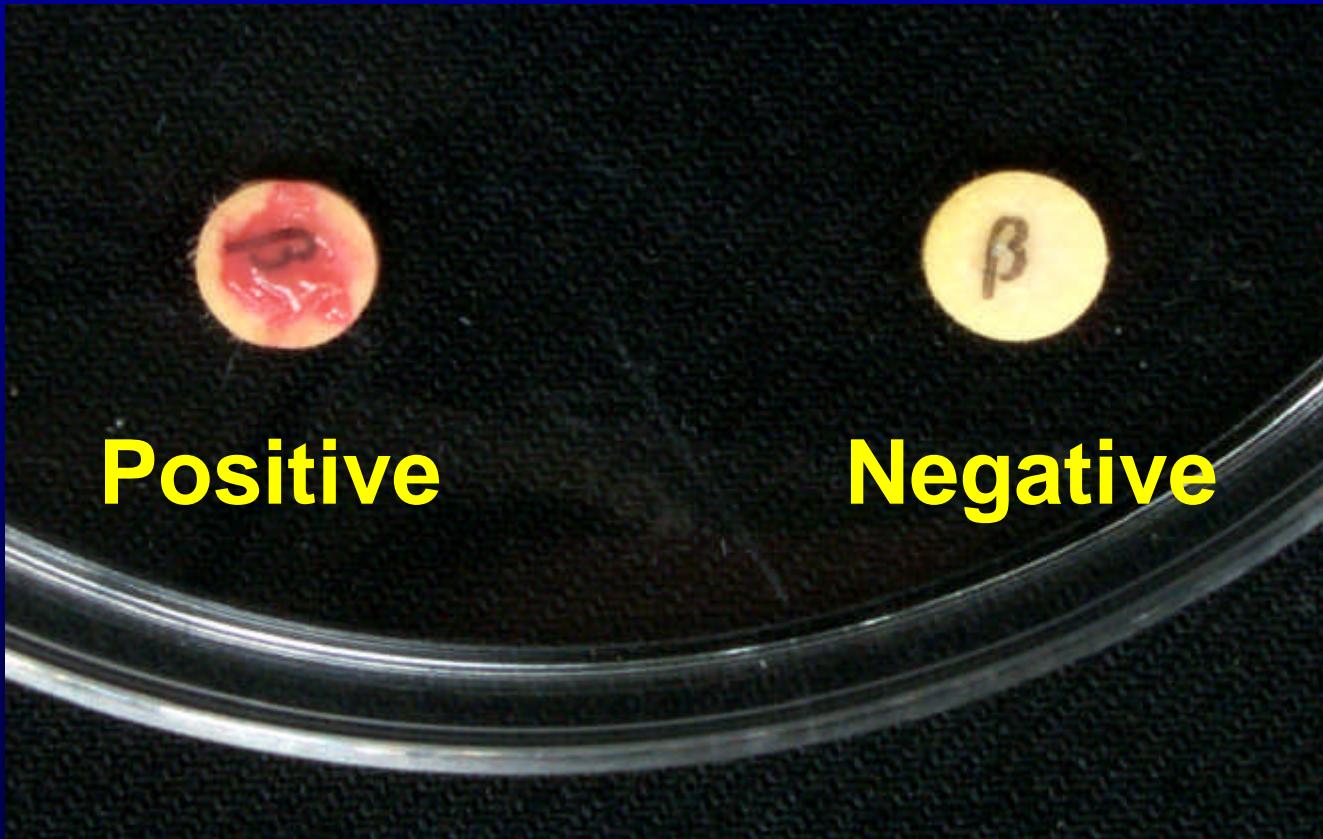
Induced β -lactamase Test

Oxacillin
(inducer)

- Sub isolate to BAP
- Test cells from periphery of zone
- Other methods



β -lactamase Test (Nitrocefin)

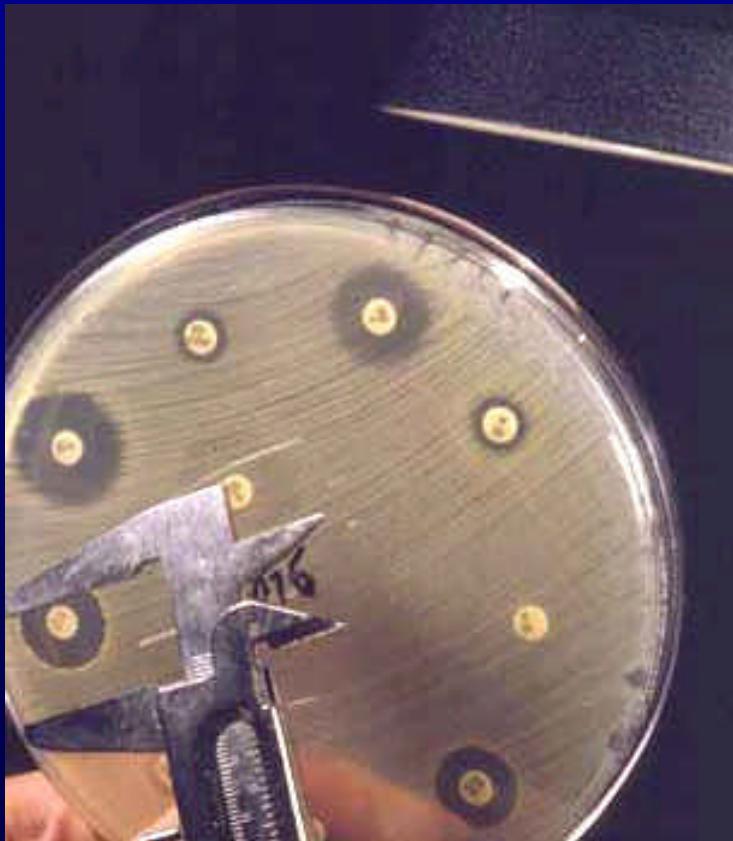


Penicillinase-Stable Penicillins

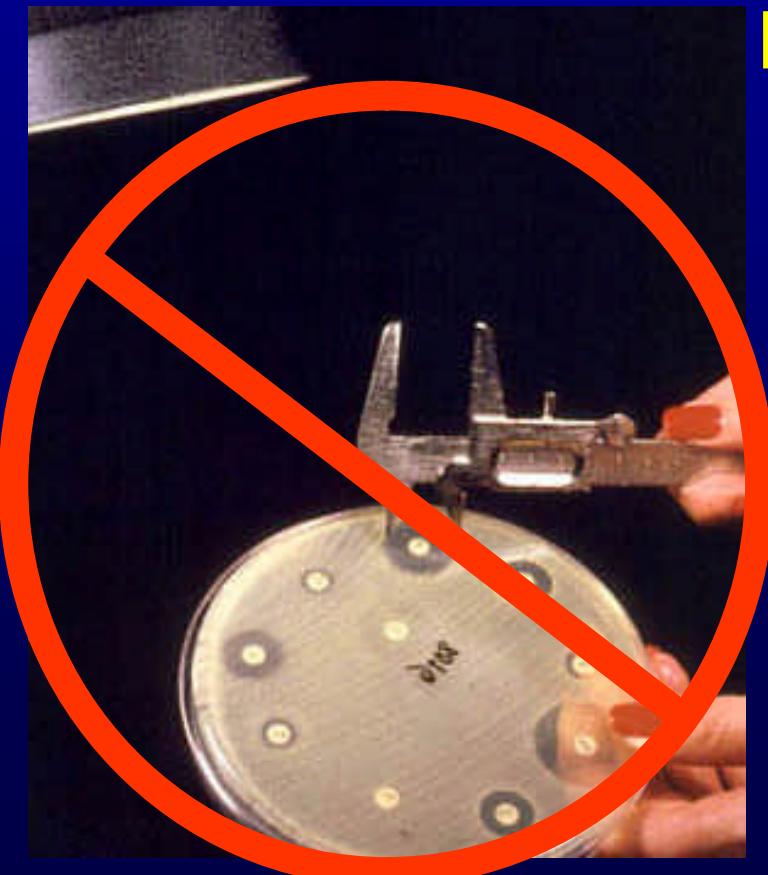
- ◆ Oxacillin = group representative
- ◆ Oxacillin performs best in in vitro test systems
- ◆ Oxacillin results used to predict results of other penicillinase-stable penicillins:
 - Nafcillin
 - Dicloxacillin
 - Methicillin

Measuring Oxacillin and Vancomycin Zones for *Staphylococcus* spp.

USE Transmitted
Light



NOT Reflected
Light

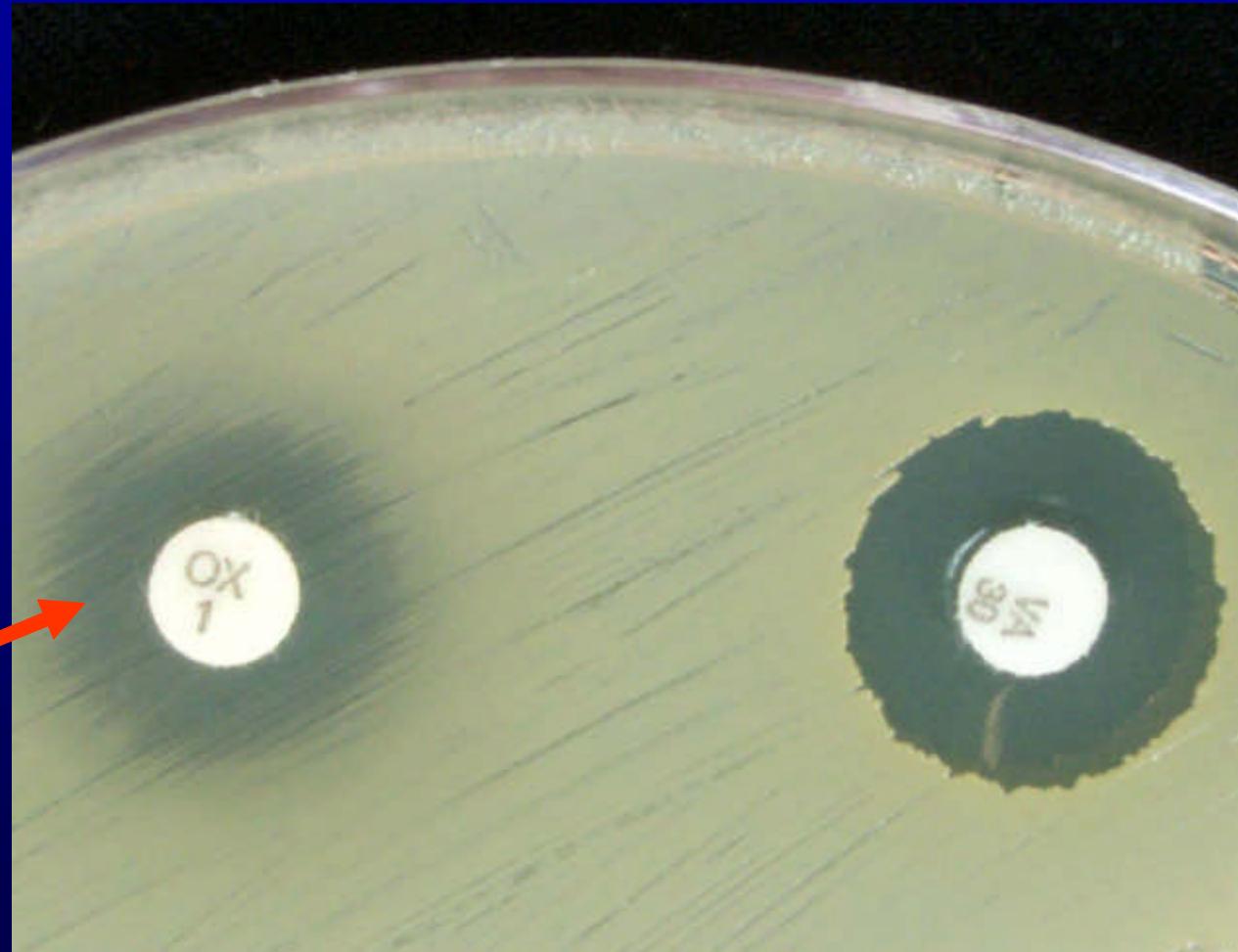


Classic MRSA - Expression

- ◆ *mecA* = genetic determinant of MRSA
- ◆ **Heterogeneous expression** - in MRSA (*mecA* +) population, some cells appear “S” and others appear “R” under standard test conditions
- ◆ **Homogeneous expression** - in MRSA (*mecA* +) population, all cells appear “R”

Heterogeneous expression

Haze within zone due
to oxacillin-R cells



Homogeneous expression

Confluent growth
up to disk

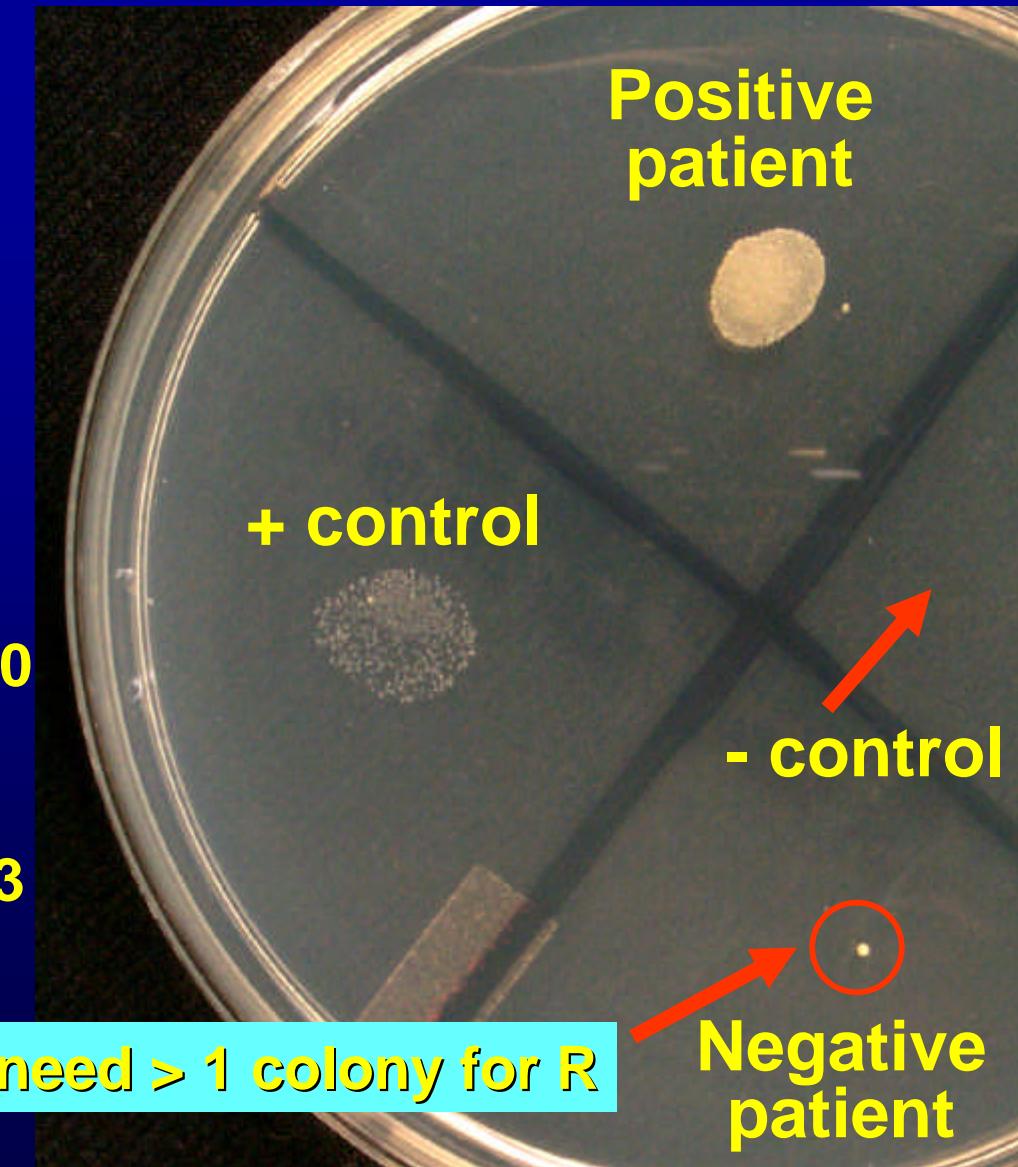


Oxacillin-salt agar screen for *S. aureus*

(MHA + 4% NaCl + 6 ug/ml oxacillin)

+ control – *S. aureus* ATCC 43300
(heteroresistant)

- control – *S. aureus* ATCC 29213



Staphylococcus spp.

“Isolates of staphylococci that are shown to carry the *mecA* gene, or that produce PBP2a, the gene product, should be reported as oxacillin resistant.”



PBP2a rapid latex agglutination assay

- Perform on isolated colonies
- Extract PBP2a
- React with latex antibody to PBP2a



Utility of *mecA* or PBP2a Tests

- ♦ **Rapid result**

- Communicate to physician rapidly to realize value of test

- ♦ **If *mecA* or PBP2a negative, physician may discontinue vancomycin**

- Empiric therapy for *S. aureus* often vancomycin if high incidence of MRSA

Utility of *mecA* or PBP2a Tests (con't)

- ◆ Usually not sufficient as a “stand alone” susceptibility test
 - Other drug results needed
 - Possible exception - MRSA surveillance cultures
- ◆ Value in previous MRSA patients?
- ◆ Good for arbitrating equivocal results from DD, MIC, or agar screen tests

Report Example (following PBP2a assay): Leg Wound Culture

GS (day 1):

Many GPC clusters

Many WBCs

Preliminary Culture Report (day 2):

Many:

***Staphylococcus aureus*, oxacillin-resistant (MRSA)**

“Additional susceptibility results to follow”

NCCLS Table 1

January 2002

NCCLS Vol. 22 No. 1

Table 1. Suggested Groupings of U.S. FDA-Approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Microbiology Laboratories

GROUP A PRIMARY TEST AND REPORT	Enterobacteriaceae ^g	<i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp. ^j	<i>Staphylococcus</i> spp.	<i>Enterococcus</i> spp. ^m
	Ampicillin ^g	Ceftazidime	Oxacillin ^k	
	Cefazolin ^a	Gentamicin	Penicillin ^k	
	Cephalothin ^a			
GROUP B ^e PRIMARY TEST REPORT SELECTIVELY	Gentamicin	Mezlocillin or ticarcillin Piperacillin		
	Amikacin	Amikacin	Azithromycin ^b or clarithromycin ^b or erythromycin	Linezolid
	Amoxicillin-clavulanic acid or ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin-clavulanic acid	Aztreonam Cefoperazone		Quinupristin- dalfopristin ^q
	Cefamandole or cefonicid or cefuroxime		Clindamycin ^b	Vancomycin ^o
	Cefepime	Cefepime	Linezolid	
	Cefmetazole ^g Cefoperazone ^g Cefotetan Cefoxitin	Ciprofloxacin	Trimethoprim- sulfamethoxazole	
	Cefotaxime ^{g, h, i} or ceftizoxime ^{g, i} or ceftriaxone ^{g, h, i}	Imipenem or meropenem	Vancomycin	
	Ciprofloxacin ^g or	Tobramycin		



***Staphylococcus* spp.**

Use Pen and Ox results to deduce results for other β -lactams

Pen	Ox	Comments
S	S	S to penicillins, cephems, carbapenems
R	S	R to β -lactamase-labile pens; S to β -lactamase-stable pens; S to β -lac / β -lac inhibitor combos, cephems, carbapenems
R	R	R to all β -lactams

Staphylococcus aureus

clindamycin	S
erythromycin	S
oxacillin	S
penicillin	R
vancomycin	S

“Cefazolin and other β -lactams (except amoxicillin, ampicillin, and penicillins) are active against oxacillin-S and penicillin-R staphylococci.”

**Consider adding comment to report
to further explain β -lactam results*

Staphylococcus aureus

clindamycin	R
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

“Oxacillin-R staphylococci are resistant to all β -lactams. MRSA isolated, please check Infection Control policies.”

Staphylococcus aureus

cefazolin	 R*
clindamycin	R
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

***If any *b-lactam* is tested and tests “S”,
do not report or change to “R” for MRSA**

Staphylococcus aureus

clindamycin	S
erythromycin	S
oxacillin	R
penicillin	R
vancomycin	S

Historically, MRSA has been multiply resistant to other anti-staphylococcal agents. However, some MRSA, particularly community-associated strains are not multiply resistant.

“Borderline” MRSA

BORSA

MRSA

Resistance	<i>mecA?</i>	Mechanism	Multiply R?
Classic	yes	PBP2a	yes*
Borderline (blac)	no	Ý b-lactamase	no
Borderline (MOD-SA)	no	mod PBPs 1,2,4	no

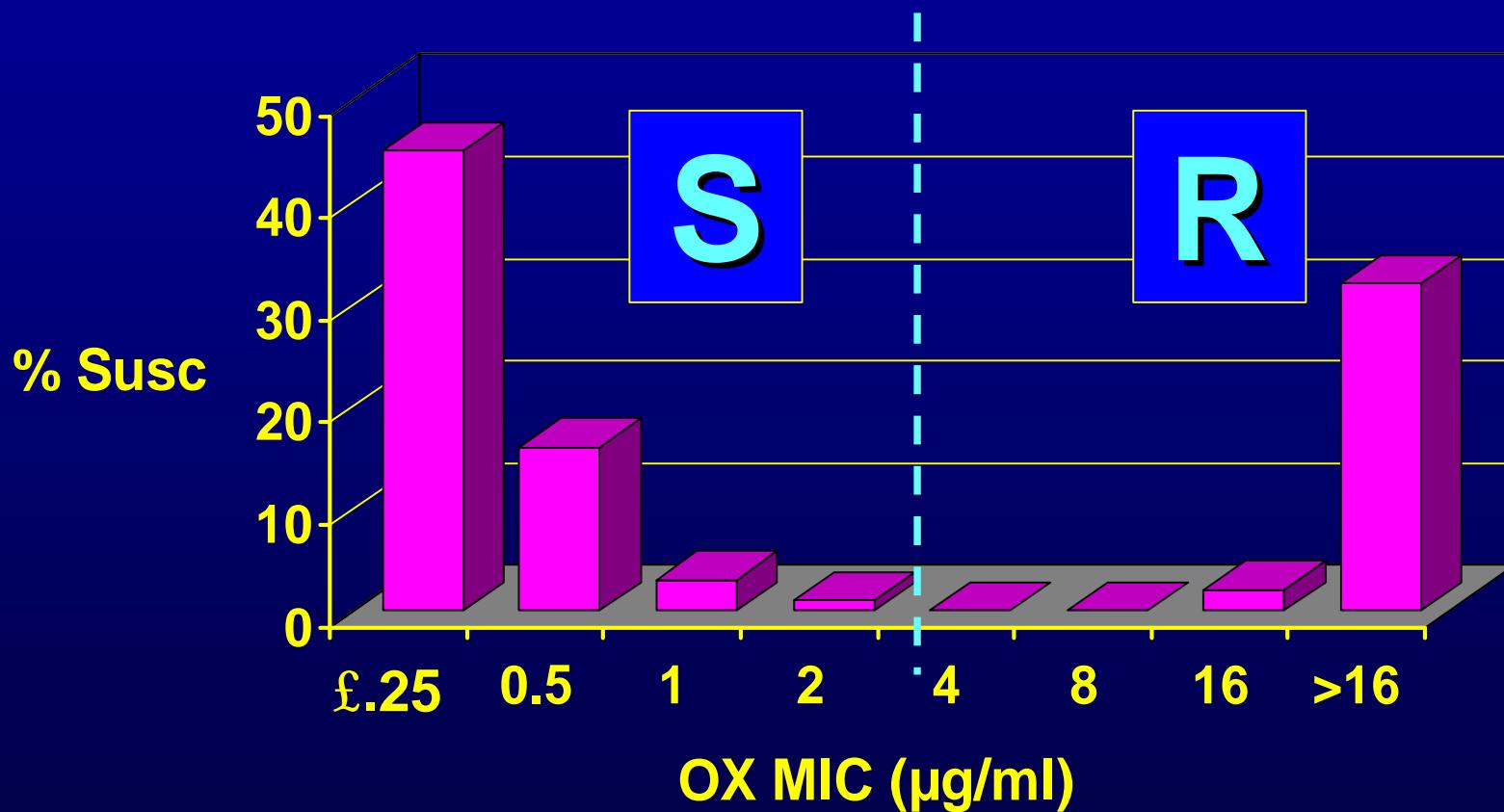
*CA-MRSA often not multiply R

Staphylococcus aureus Oxacillin Breakpoints

	MIC (mg/ml)		
	S	I	R
<i>S. aureus</i>	≤ 2	-	≥ 4

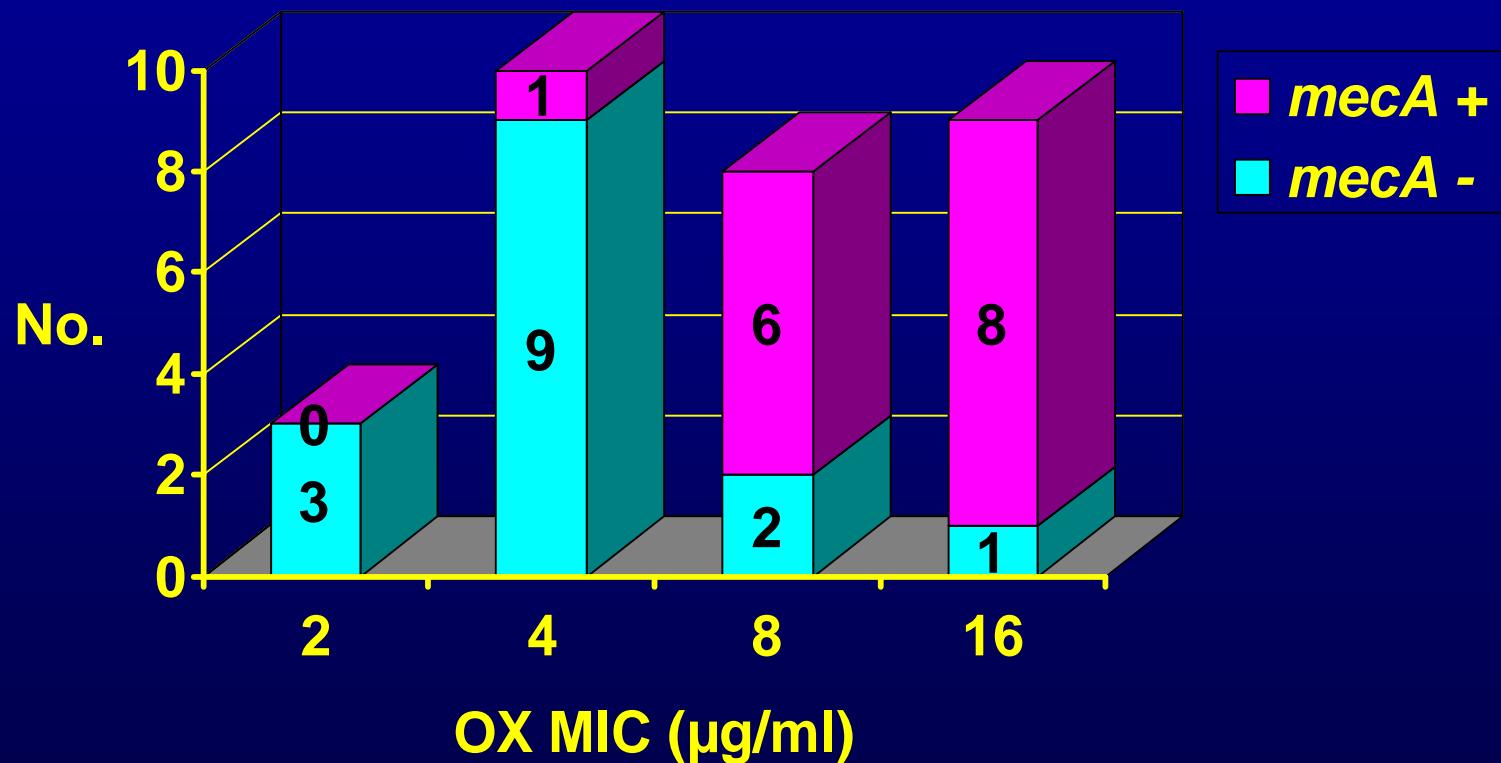
NCCLS M100-S13 (M7); Table 2C

Oxacillin MIC ($\mu\text{g/ml}$) vs. *S. aureus* UCLA (n=4192)



mecA PCR vs. Oxacillin MIC ($\mu\text{g/ml}$)

S. aureus (n=30)



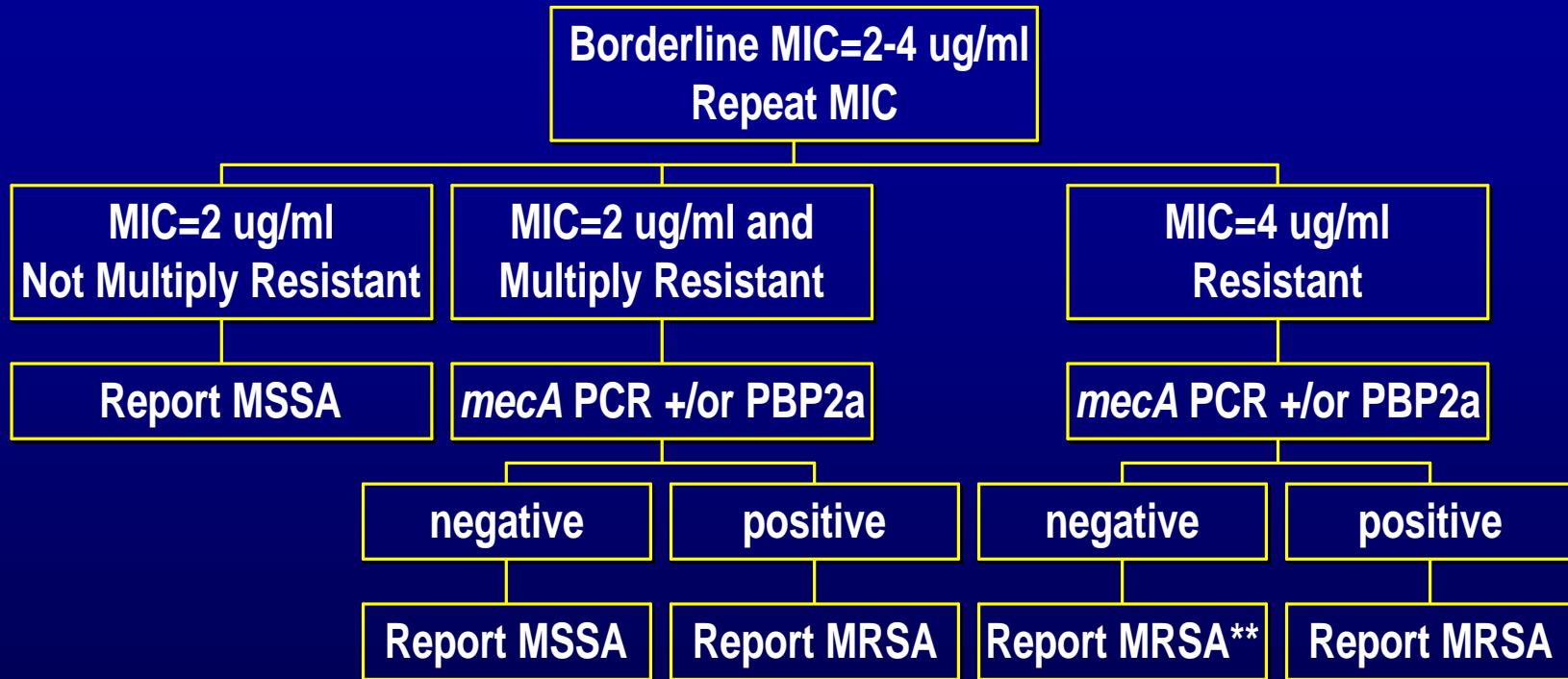
UCLA unpublished data
Isolates collected 1995-2001

“Borderline” MRSA (*mecA* neg) Therapy

- ◆ Can oxacillin be used?
- ◆ Literature suggests β -lactamase inhibitor combination or other β -lactam might be effective.

Pefanis, et al. 1993. AAC. 37:507.

“Borderline” MRSA Workup



*Add report comment: “*mecA* (or PBP2a)
negative” Discuss with physician.

Staphylococcus aureus **(*mecA* neg)**

MIC ($\mu\text{g/ml}$)

cefazolin	1S R
clindamycin	0.5 S
erythromycin	0.5 S
oxacillin	4 R*
penicillin	R
vancomycin	0.5 S

**“*Atypical oxacillin resistance (*mecA* negative);
Infectious Diseases consult suggested.”**

S. aureus
Clindamycin

Clindamycin

***Staphylococcus* spp.**

Erythromycin/Clindamycin

Mechanism	Determinant	Erythro	Clinda
Efflux	<i>msrA</i>	R	S
Ribosome alteration	<i>erm</i>	R	(R)*

msrA = macrolide streptogramin resistance

erm = erythromycin ribosome methylase

*may test resistant or may test susceptible and require induction to show resistance)

Staphylococcus spp. *erm*-mediated Resistance

- ♦ *erm* confers resistance to macrolide, lincosamide, streptogramin B (MLS)
- ♦ MLS resistance occurs via methylation of the 23S rRNA and reduces binding of MLS agents to the ribosome
 - **MLS_Bi** = inducible resistance to lincosamide (clindamycin); “D test” required
 - **MLS_Bc** = constitutive resistance to lincosamide; shows clindamycin resistance in routine ASTs

Staphylococcus aureus

clindamycin	S
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

*Is this phenotype due to:
msrA and truly clindamycin S?
erm with inducible clindamycin R?*

Staphylococcus aureus

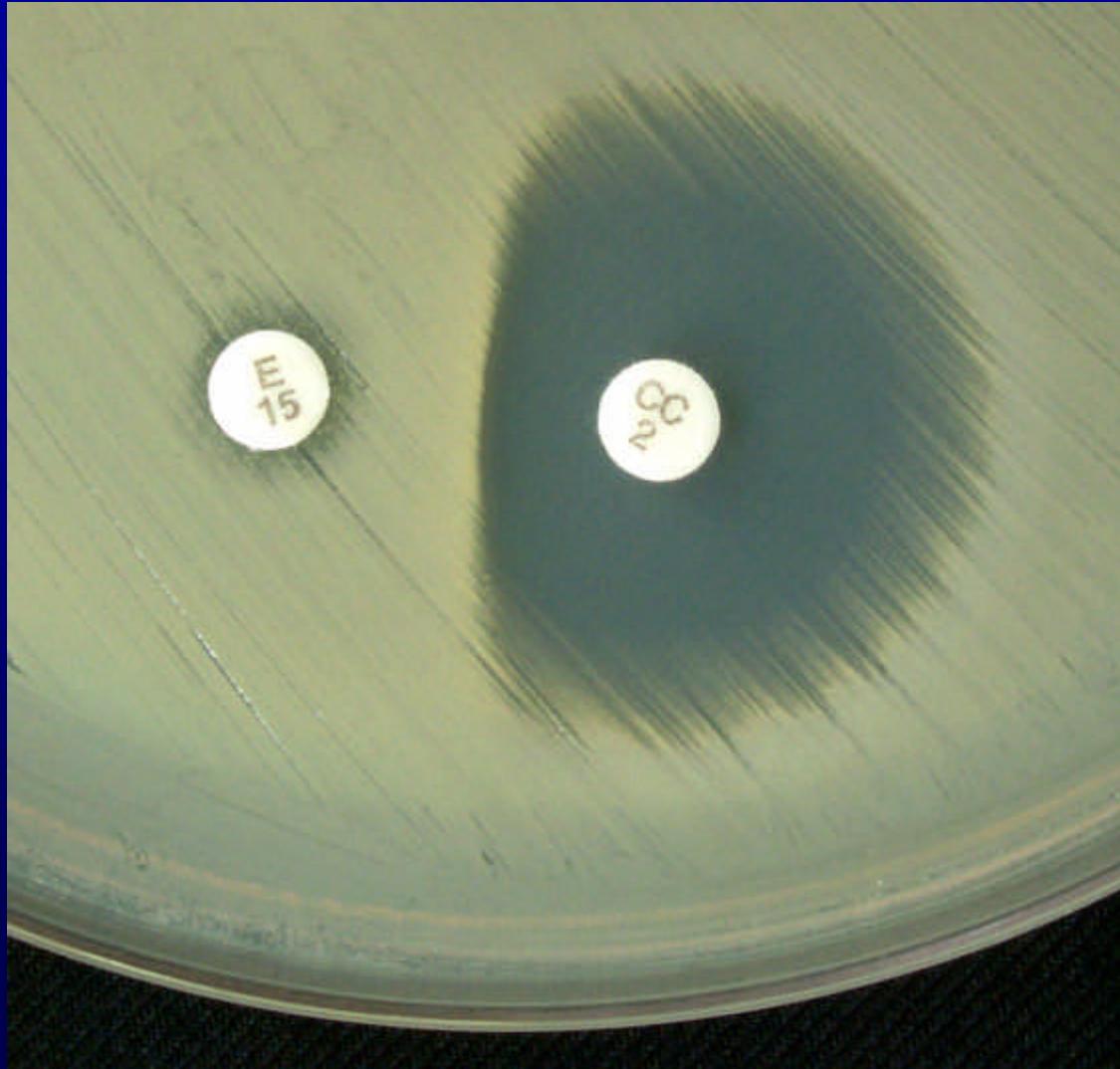
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

“Contact laboratory if clindamycin results needed”

If clindamycin-S, erythromycin-R, do not report as clindamycin-S without performance of “D Test”

“D Test”

Inducible
Clindamycin
Resistance
(*erm*-mediated)



“D Test” positive

Staphylococcus aureus

erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

“This *S. aureus* demonstrates inducible clindamycin resistance in vitro and isolate may develop clindamycin resistance during therapy”

“D Test” negative

Staphylococcus aureus

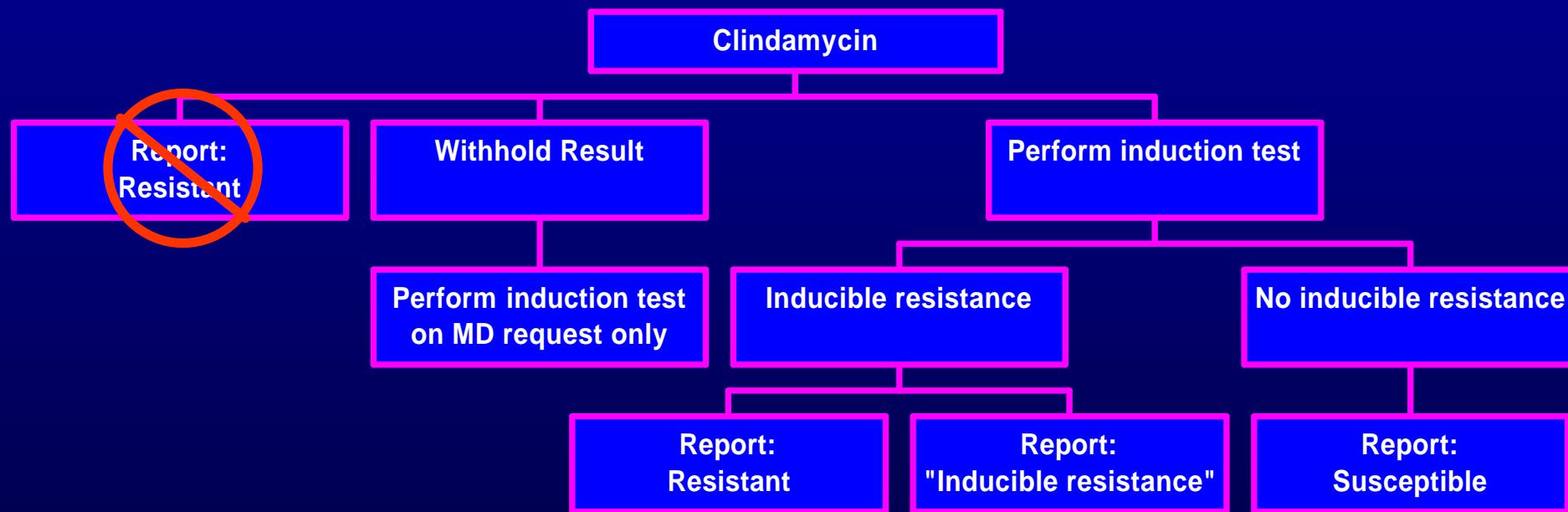
clindamycin	S
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

“This *S. aureus* DOES NOT demonstrate inducible clindamycin resistance in vitro”

Clindamycin Test/Report Options

Staphylococcus spp.

Erythromycin-R & Clindamycin-S



Staphylococcus aureus

clindamycin	S
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

Many community-associated MRSA are clindamycin-S (msrA type) and clindamycin is a therapy option.

VISA / VRSA

Vancomycin-intermediate *Staphylococcus aureus*
Vancomycin-resistant *Staphylococcus aureus*

VISA VRSA

Staphylococcus spp. Vancomycin

MIC (μ g/ml)

Susceptible £ 4

Intermediate 8 - 16

Resistant 3 32

VISA = (4) - 16 μ g/ml

VRSA = 3 32 μ g/ml

NCCLS M100-S13; Table 2C

Detection of VISA / VRSA

◆ Disk diffusion

- VISA – not detected; MIC of 8 µg/ml are “S” by DD (even at 24 h)
- VRSA - “R”; use transmitted light

◆ MIC

- VISA, VRSA - generally, overnight broth microdilution systems detect both
- VISA or VRSA – Vitek, MicroScan may not detect (missed 2nd VRSA)
- Check *S. aureus* with vanco MIC \geq 4 µg/ml

Detection of VISA / VRSA (con't)

- ♦ Brain heart infusion (BHI) agar with 6 µg/ml vancomycin*
 - VISA and VRSA grow on this
 - Incubate 24 h
 - QC
 - *S. aureus* ATCC 29213
 - *E. faecalis* ATCC 51299
 - Consider use if routine test method has been shown to miss VISA / VRSA

*medium used for VRE screening

Detection of VISA

	<u>MIC (μg/ml)</u>
♦ MicroScan ON	8
♦ Etest	6-8
♦ Sensititre	4,8
♦ Vitek	4
♦ BHI-Van (6 μ g/ml)*	growth
♦ MicroScan rapid	£2, ³ 16
♦ Disk diffusion	inadequate

*medium used for VRE screening

Tenover, et al. 1998. JCM. 36:1020

VISA

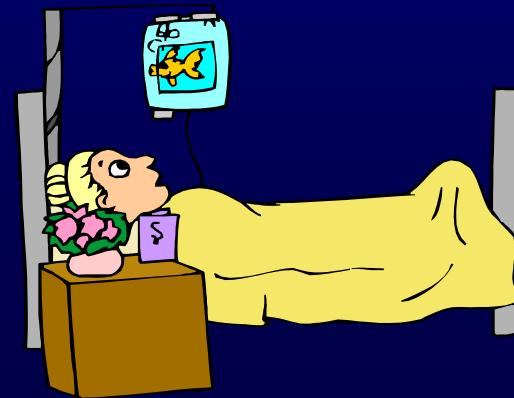
- ◆ 8 cases to date in USA
- ◆ Pts. previously had MRSA
- ◆ Pt. previously treated with vancomycin
- ◆ Most are MRSA

Fridkin et. al. 2001. Clin. Infect. Dis. 32:108.
Fridkin et. al. 2003. Clin. Infect. Dis. 36:429.

Case Study

VISA - Pt. JB (UCLA)

- ◆ 27 y.o. referral patient
- ◆ Cholecystitis
- ◆ Bile drainage (liver abscesses)
- ◆ Culture results
 - 2 strains VISA
 - MRSA (vancomycin-S)
 - *S. maltophilia*



Case Study - VISA Pt. JB

Strain	<i>mecA</i>	MIC (mg/ml)	
		OX	Van
1	-	0.5	8
2	+	>16	8

**VISA colonies may be
smaller and slower growing
than typical *S. aureus* as
shown on this 48h BAP**

VISA

MRSA (not VISA)



Marlowe, et al. 2001. JCM. 39:2637.

VRSA – 1st Case

Michigan June 2002

- ◆ 40 y.o. female, diabetic, hemodialysis
- ◆ Pt. previously had MRSA and VRE (*vanA*)
- ◆ Pt. previously treated with vancomycin
- ◆ VRSA isolated from catheter exit site and foot wound
- ◆ Wounds healed at 3 months
- ◆ VRSA had *mecA* and *vanA*

MMWR. 2002; 51:565-7.

Chang et al. 2003. NEJM 348:1342

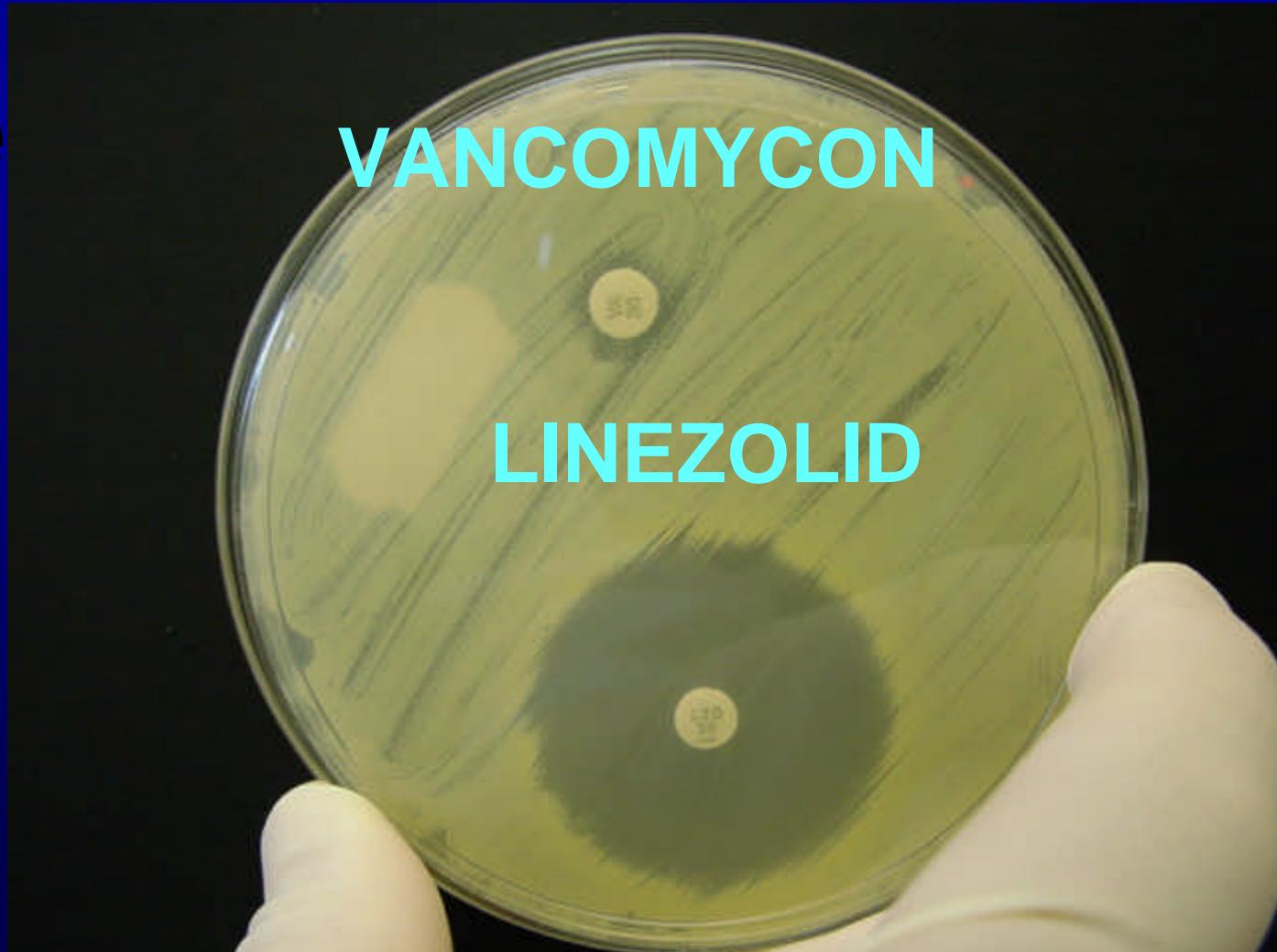
VRSA – 1st Case

Michigan June 2002 (con't)

- ◆ Routine vancomycin tests (NCCLS methods)
 - MIC = 1024 µg/ml
 - DD = no zone (or heavy haze within zone)
 - Screen plate (BHI + 6 µg/ml vancomycin) = growth
- ◆ Resistance detected by automated systems (MicroScan, Vitek)
- ◆ “S” to chloramphenicol, linezolid, minocycline, quin-dalfo, TMP-SMZ

VRSA 1st Case

From Michigan
Health Dept. Lab



VRSA – 2nd Case

Pennsylvania September 2002

- ◆ 70 y.o. male, morbid obesity
- ◆ Chronic foot ulcer grew VRSA
- ◆ Routine vancomycin tests (NCCLS methods)
 - MIC = 32 µg/ml
 - DD = 12 mm
 - Screen plate (BHI + 6 µg/ml vancomycin) = growth
- ◆ Not detected by automated systems (MicroScan, Vitek)
- ◆ “S” to chloramphenicol, linezolid, minocycline, quin-dalfo, rifampin, TMP-SMZ

VRSA 2nd Case

From CDC Lab



Confirmation of VISA / VRSA

If you suspect a VISA / VRSA:

- 1. Repeat ID and susceptibility tests**
- 2. Contact your institution's infection control department**
- 3. Contact CDC at SEARCH@cdc.gov**
- 4. Contact your local health department**
- 5. SAVE ISOLATE!!**

S. aureus AST QA/QC/Competency

QA/QC

QC of AST for *S. aureus* NCCLS Recommendations

◆ Disk diffusion and MIC tests

- *S. aureus* ATCC 25923 (DD); *S. aureus* ATCC 29213 (MICs)
- *E. coli* ATCC 35218 (β -lactam/ β -lactamase inhibitor combos)
- QC daily; weekly (if meet acceptable daily criteria)

◆ Oxacillin-salt agar screen

- *S. aureus* ATCC 43300
- *S. aureus* ATCC 29213
- QC each day of use

Supplemental QA/QC of AST for *S. aureus*

- ◆ **Verify AST results on patient's isolates**
 - NCCLS M100-S13 (2003) - **Verification Tables** (Table 4 - disk diffusion, Table 8 - MIC)
- ◆ **Assess competency of staff**
- ◆ **Proficiency surveys**
- ◆ **Antibiogram review**
- ◆ **Other**

Verifying Results

S. aureus

- ◆ All labs should verify the following results for *S. aureus*:
 - Linezolid – non-susceptible
 - Quinupristin-dalfopristin – I or R
 - Vancomycin – I or R
- ◆ Confirm with NCCLS dilution reference method

***Staphylococcus* spp.**

Linezolid

	<u>Susc</u>	<u>Int</u>	<u>Res</u>
DD (mm)	≥21	-	-
MIC ($\mu\text{g/ml}$)	£4	-	-

***investigate any non- “S” isolate**

..Repeat ID and AST

..Save isolate

..Send to reference lab

“Verify” Results Patient’s Isolates - HOW?

- ◆ Check transcription
- ◆ Reexamine plate/tray, purity plate, etc.
- ◆ Check previous isolates on patient

THEN.....

- ◆ Confirm ID and/or
- ◆ Repeat AST (alternate method?)
- ◆ Get assistance from reference lab

Verifying MRSA

- ♦ NCCLS suggests verification if you feel this is appropriate for **your institution**
- ♦ ..from JH..consider verifying MRSA when isolated from **new patient** (first time MRSA for that patient)

Why Verify MRSA?

- ♦ **Clinical consequences** of reporting MRSA are significant
 - Isolation of patient
 - Broad spectrum (e.g. vancomycin) therapy
 - “MRSA” label
- ♦ ***S. aureus* is often present in culture with oxacillin-resistant organisms (e.g. enterococcus, coagulase-negative staphylococci)** – increases risk for **mixed susceptibility tests**

Verify ?*

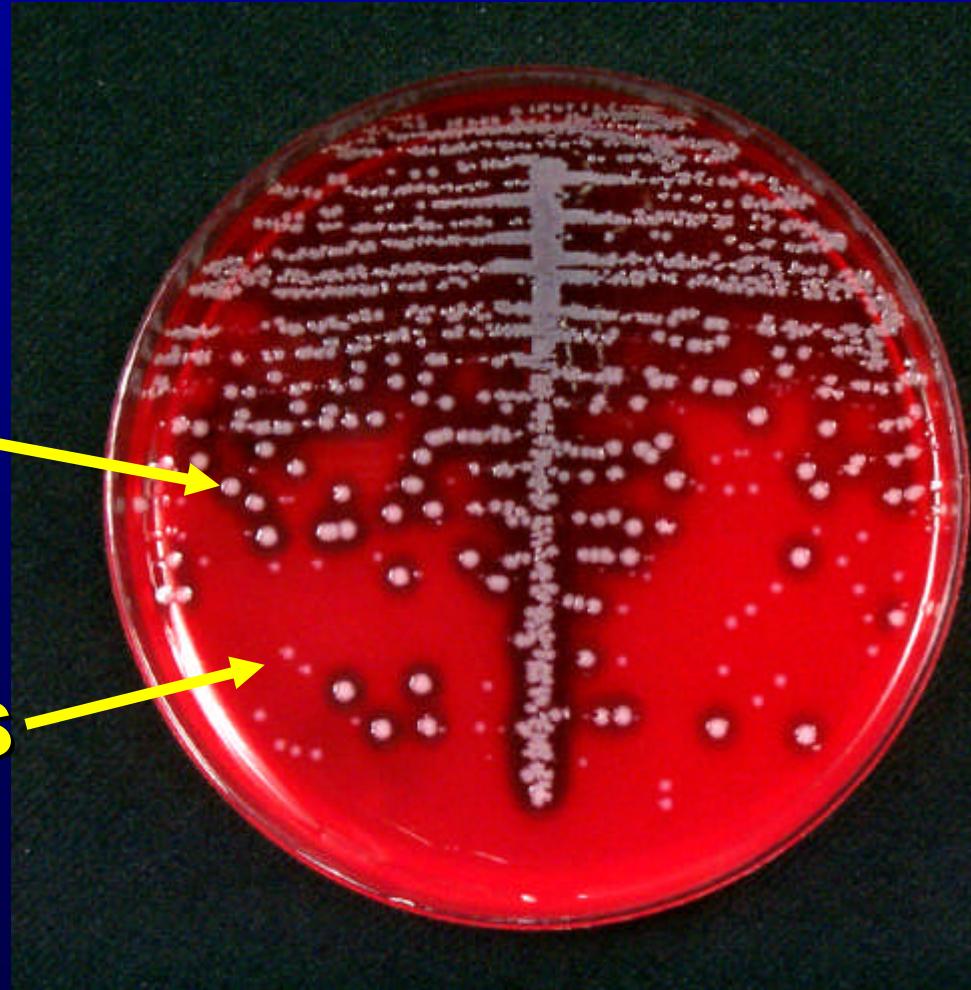
Staphylococcus aureus

MIC (μ g/ml)

clindamycin	>8 R
erythromycin	>8 R
oxacillin	>16 R
penicillin	R
vancomycin	1 S

***Institution Specific (NCCLS M100-S13)**

**Examine test closely -
MIC purity plate on patient's *S. aureus***



MSSA
Enterococcus

FINAL Report*

Staphylococcus aureus

MIC (μ g/ml)

clindamycin	£0.5 S
erythromycin	£0.5 S
oxacillin	£0.5 S
penicillin	R
vancomycin	1 S

*After repeat MIC test without enterococcus
contaminating the inoculum.*

S. aureus
Susceptibility Statistics
(Annual Antibiogram)

STATS

NCCLS M39-A Guideline

“Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data”

May 2002



M-39A Recommendations

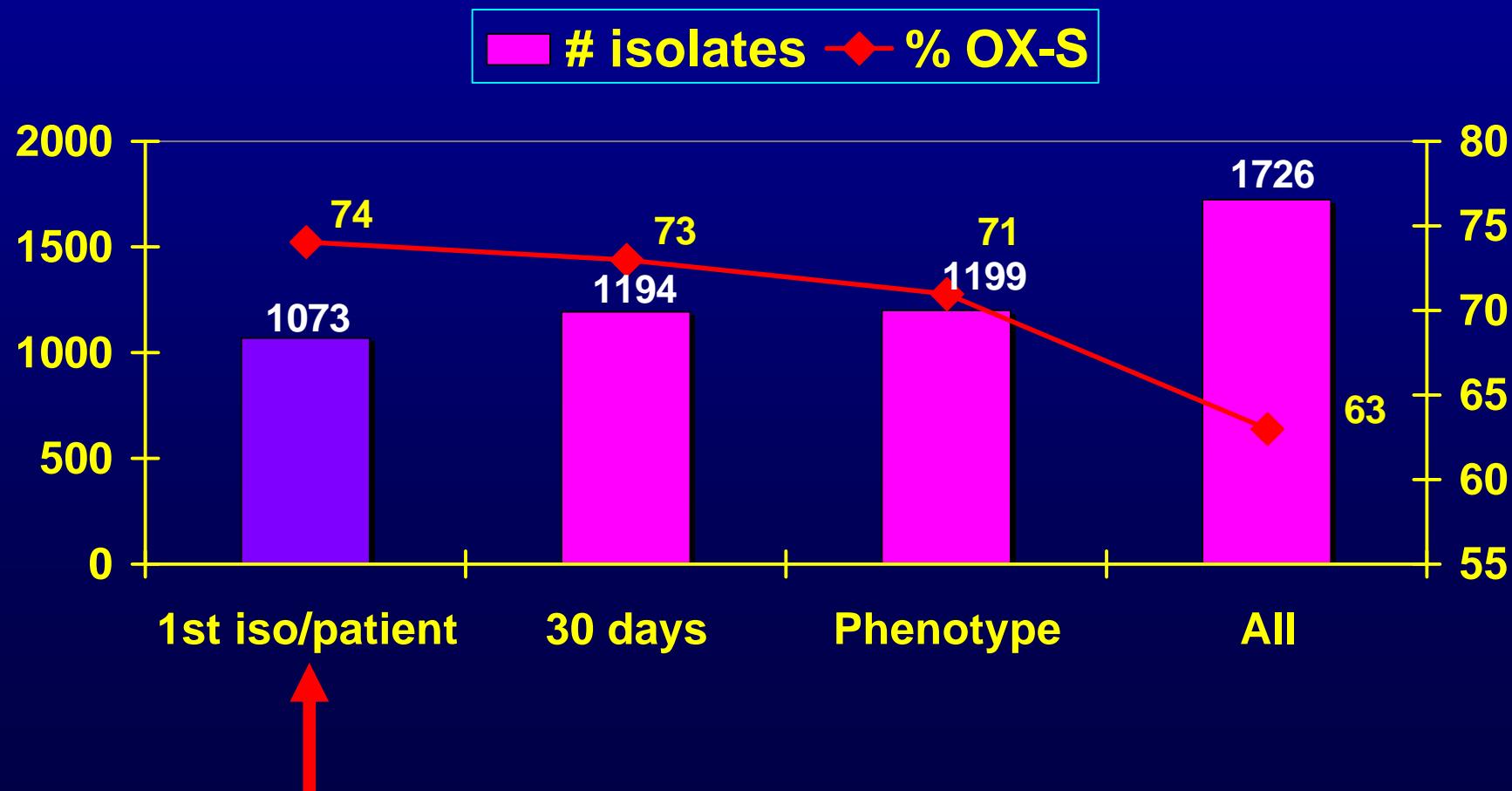
- ◆ Analyze/present data **annually**
 - Sufficient to **GUIDE EMPIRIC THERAPY**
- ◆ Calculate **%S** (do not include %)
- ◆ Include **diagnostic** (not surveillance) isolates
- ◆ Include the **1st isolate/patient**, irrespective of
 - body site
 - antimicrobial pattern
- ◆ **For *S. aureus* present all and MRSA subset**

Handling Repeat Isolates Options

- ◆ Count 1st isolate/patient/year
 - Irrespective of body site or overall susceptibility profile
 - Count each patient once
 - recommended in NCCLS M39-A
- ◆ Count repeat isolates after 30 days have elapsed since testing the previous isolate from a given patient
- ◆ Count repeat isolates that have different phenotypes or different results for one (or more drugs (e.g., S to R, R to S)

Example of %S for oxacillin when analyzing a single data set (N=1726) and eliminating duplicates 3 ways.

S. aureus - Oxacillin



Example: US Hospital Antibiogram 2002

	No.	clin	% Susceptible				
			ery	ox	pen	t-s	van
all SA	1073	80	50	74	9	97	100
MRSA	449	34	4	0	0	94	100

Summary

- ◆ Use the most **current NCCLS standards** (new tables published every January).
- ◆ Know **specific protocols** for detecting:
 - Heteroresistant MRSA (with *mecA*)
 - Borderline MRSA (without *mecA*)
 - Inducible clindamycin resistance
 - VISA, VRSA

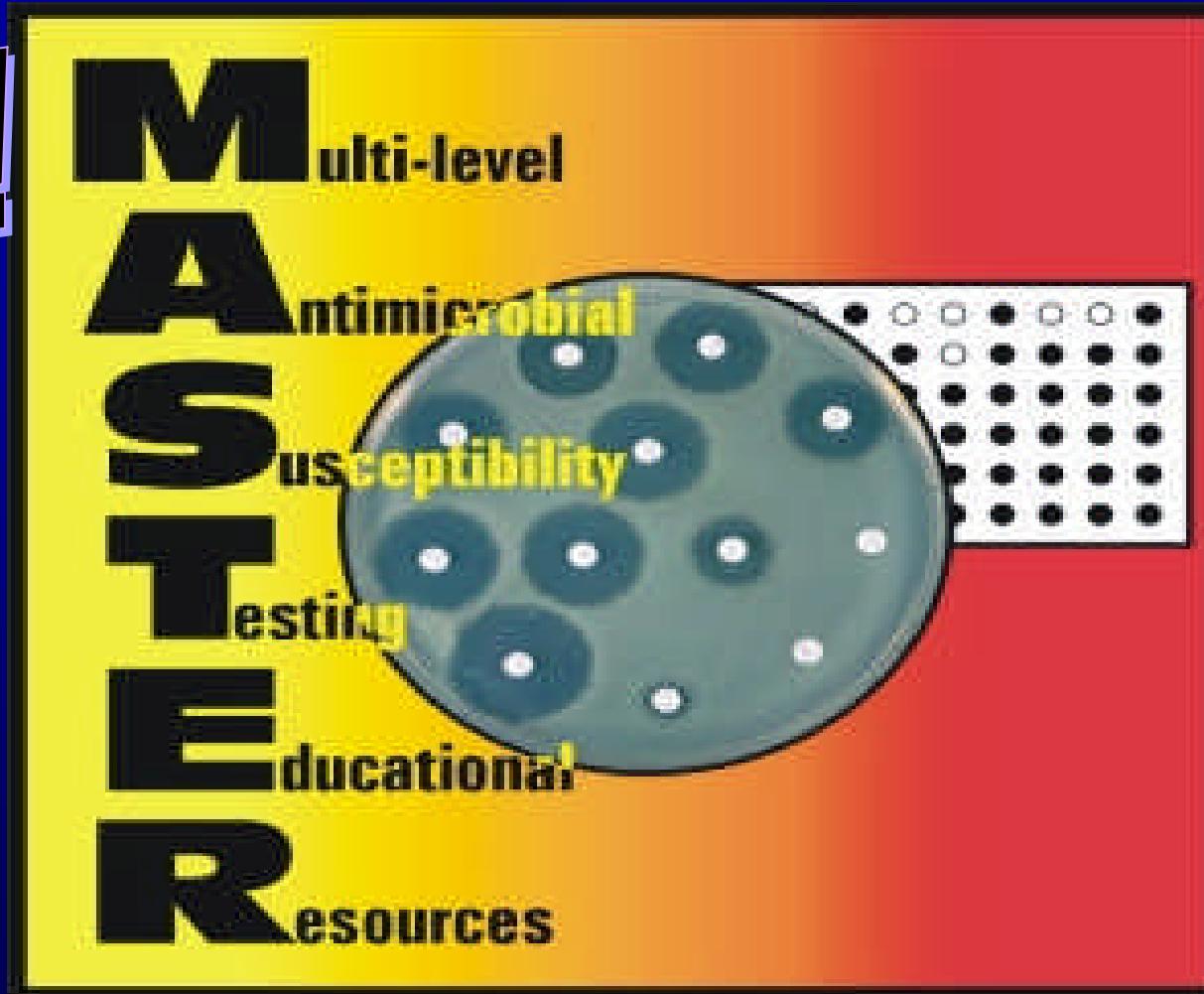
Summary

- ◆ Make certain your **reports** are clearly understood by your physicians.
- ◆ Test and report **supplemental agents** on *S. aureus* based on the needs of your physicians.
- ◆ Without delay, follow up on potential **VISA** and **VRSA** with infection control and your public health department.

Summary (con't)

- ◆ **Verify** all patient results. This may require retesting to confirm ID and AST (e.g., first time MRSA; VISA; VRSA)
- ◆ Develop a comprehensive **QA/QC** program for testing *S. aureus* in your laboratory
 - Include assessing competency of staff
 - Carefully review annual antibiogram
- ◆ If using a **commercial AST system**, regularly review the product literature provided by the manufacturer.

Thank you!



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